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A Study of Assessing the Impact of Pantohematogen, Embryotoxicity, and Teratogenicity

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ABSTRACT

This study investigated whether pantohematogen has any embryotoxic or teratogenic effects. Pantohematogen is a bioactive substance obtained from the velvet antlers of the *Altai wapiti* and is used in confectionery products. A total of 180 female Wistar rats were exposed to different doses of the test compound. During the experiment, no significant changes in behavior or appearance were detected. The results showed that pantohematogen did not impair fertility or elevate embryonic loss. However, female offspring from dams that received a subtoxic dose showed delayed puberty, while those exposed to lower doses did not show such an effect. Overall, the findings suggest that pantohematogen is unlikely to have harmful effects and is safe for use as a beneficial ingredient.

Keywords: Deer antler, Dietary supplement, Effectiveness, Safety, Pantohematogen

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Introduction

For centuries, adaptogenic substances have played a significant role in herbal medicine, and recently, antler-based products containing pantohematogen have gained increasing attention [1-9].

Given this growing popularity, assessing the safety of pantohematogen is crucial. It is derived from the velvet antlers of the *Altai wapiti* in Russia. Previous studies have highlighted the beneficial properties of velvet antlers, including their anti-inflammatory effects, immune-supporting potential, and ability to alleviate stress [10-13].

This study focuses on evaluating whether pantohematogen, commonly incorporated into various confectionery products as a useful ingredient, exhibits any embryotoxic or teratogenic effects.

Materials and Methods

This research was conducted at Regenerative Medicine, Tomsk National Research Medical Centre of the Russian Academy of Sciences, and the Goldberg Research Institute of Pharmacology.

Assessment of pantohematogen's impact on prenatal development

A total of 180 Wistar rats which were female were chosen and divided into groups of 14-17 individuals. The females were paired with males at a 2:1 ratio. The finding of spermatozoa in a vaginal smear was planned for the first day of gestation. The experimental solution administered consisted of pantohematogen (10 parts), glucose (20 parts), and ascorbic acid (1 part) and was delivered via gastric injection once per day. Control groups received a solution with glucose and ascorbic acid but without pantohematogen.

Three different dosages were evaluated: a subtoxic level (1550 mg/kg, with 500 mg/kg of pantohematogen), an intermediate level (775 mg/kg, with 250 mg/kg of pantohematogen), and a therapeutic level (155 mg/kg, with 50 mg/kg of pantohematogen). The control solution was provided in corresponding doses of 1050, 525, and 105 mg/kg. Observations of weight, appearance, and behavior were conducted on Days 1, 7, and 14.

On Day 20, euthanasia was performed through manual cervical dislocation and reproductive parameters were examined, including the number of corpora lutea, implantation sites, and both viable and non-viable fetuses. Pre- and post-implantation loss rates were calculated. Fetal assessments included weight measurements, macroscopic analysis, cranio-caudal length measurement, and sex identification. Half of the collected fetuses were preserved in Bouin's solution for detailed internal organ analysis using Wilson's method, while the remaining samples were prepared with Dawson's staining technique for skeletal evaluation. Statistical analysis was conducted based on litter-level data, meaning findings from each female rat were treated as independent observations.

Assessment of pantohematogen's impact on postnatal development

To evaluate postnatal outcomes, pregnant rats in the test group were given a daily dose of 1550 mg/kg of the experimental solution from Day 6 until birth, while control animals received 1050 mg/kg of glucose and ascorbic acid. Maternal weight, behavior, and general health were recorded throughout pregnancy. On Day 18, individual cages were prepared for the approaching deliveries. After birth, parameters such as litter size, delivery date, and offspring sex distribution were documented.

24 hours after birth, four males and four females were taken from each litter, and the mother rats were taken away between Days 25 and 30. The developmental progress of the pups was tracked, including body weight changes, physical development, motor function, and behavioral responses. The Open Field Test was utilized to assess their general and exploratory activity, while learning ability (CRPI) and stress adaptation were also analyzed.

Assessment of pantohematogen's impact on fertility

To examine the potential effects on reproductive capacity, two separate studies were conducted, involving a total of 60 male and 190 female rats.

First experimental study: effects on female reproduction

For 15 days, female rats in the experimental group were administered the experimental solution at doses of 1550, 775, and 155 mg/kg, while control animals received 1050 mg/kg of a glucose and ascorbic acid solution. Following this treatment phase, the females were housed with intact males at a 2:1 ratio for 10 days. Pregnancy detection was confirmed through vaginal smear analysis.

Between Days 17 and 20 of gestation, a subset of pregnant females was euthanized via manual cervical dislocation for reproductive assessment. Parameters analyzed included the number of corpora lutea, implantation sites, and viable fetuses, while pre- and post-implantation mortality rates were determined. The remaining pregnant rats were allowed to give birth naturally, and their offspring were monitored to evaluate developmental milestones and survival rates.

Second experimental study: effects on male fertility

Male Wistar rats in the experimental group were given a daily dose of 1550 mg/kg of the test solution for a total of 60 days. The control group received an equivalent dosage (1050 mg/kg) of glucose and ascorbic acid. On the 61st day, the males were individually housed with two untreated female rats for a 10-day mating period, during which vaginal smear analysis was used to confirm pregnancies.

As in the first experiment, between Days 17 and 20 of gestation, a portion of pregnant females was euthanized for reproductive examination. The number of corpora lutea, implantation sites, and viable fetuses were recorded, and fertility indices, as well as pre and post-implantation mortality rates, were analyzed. The remaining females delivered their litters naturally, and postnatal development and survival rates of the pups were monitored.

Histological analysis of male reproductive organs

Following the 60-day treatment period, male Wistar rats were euthanized, and their testes were collected and preserved in Carnoy's solution. Tissue samples, sectioned at 5-7 μm thickness, were processed using paraffin embedding and stained with hematoxylin and eosin for histological evaluation.

Statistical analysis

The collected data were analyzed using statistical methods, including the Student's t-test, the Mann-Whitney U test, and Fisher's angular transformation. Experimental results were compared against control values as well as historical control data (HCD) to ensure reliability and accuracy.

Results and Discussion

No visible alterations in physical appearance or behavioral patterns were observed in any of the test groups. The pattern of body weight fluctuations in the rats administered the test solution from day 1 to Day 6 and from Day 6 to Day 16 (subtoxic dose), as well as from day 6 to Day 16 (intermediate dose), was comparable to that of the control group. However, when evaluating weight gain trends against historical control data (HCD), a lower increase in body weight was noted in the first 2 weeks of pregnancy among those in the control group receiving glucose and ascorbic acid between day 1 and day 6 **(Table 1)**.

In contrast, the group that received the therapeutic dose exhibited a slight reduction in weight gain between day 7 and day 14 compared to both the control and HCD groups, though this variation did not significantly affect total pregnancy weight gain.

Furthermore, administering the subtoxic dose from day 16 to day 20 resulted in lower weight gain compared to both the control group and HCD. It is also worth mentioning that weight gain in the control group during this period was slightly lower than the HCD values, suggesting that the control treatment itself may have played a role in influencing weight dynamics.

Table 1. The dynamics of body weight change during pregnancy.

Doses and Periods	Days 1-7	Days 7-14	Days 14-20	Days 1-20
HCD	34.41 ± 3.32	26.47 ± 2.60	35.88 ± 1.56	96.18 ± 4.90
Control group (1050 mg/kg, days 1-6)	25.36 ± 2.13*	15.71 ± 3.05*	45.36 ± 2.06*	87.50 ±3.13*
Test group (1550 mg/kg, days 1-6)	20.67 ± 2.57	20.33 ± 2.51	40.00 ± 2.63	87.00 ± 4.19
Control group (1050 mg/kg, days 6-16)	25.14 ± 1.87	27.14 ± 2.44	39.64 ± 4.01	88.21 ± 6.54
Test group (1550 mg/kg, days 6-16)	22.00 ± 4.01	25.31 ± 2.21	41.56 ± 2.40	81.25 ± 6.25
Control group (525 mg/kg, days 6-16)	31.67 ± 1.18	30.00 ± 3.23	30.00 ± 4.25	91.67 ± 6.07
Test group (775 mg/kg, days 6-16)	15.71 ± 3.09	29.64 ± 3.25	33.93 ± 3.42	79.29 ± 4.86
Control group (105 mg/kg, days 6-16)	30.56 ± 2.27	32.22 ± 2.90	38.89 ± 2.98	100.56 ± 3.48
Test group (155 mg/kg, days 6-16)	34.64 ± 2.15	15.00 ± 1.62***	42.00 ± 5.12	91.67 ± 5.81
Control group (1050 mg/kg, days 16-20)	32.31 ± 3.61	30.77 ± 1.86	30.38 ± 2.00*	93.46 ± 3.86
Test group (1550 mg/kg, days 16-20)	37.5 ± 2.15	34.6 ± 3.72	21.43 ± 3.16***	88.93 ± 5.50
Control group (525 mg/kg, days 16-20)	22.22 ± 2.65	16.11 ± 1.62	49.00 ± 3.00	68.33 ± 5.27
Test group (775 mg/kg, days 16-20)	21.00 ± 2.77	32.00 ± 1.86	33.00 ± 3.89	86.00 ± 5.57

The administered doses and the timing of injections had no impact on the ratio of sex of the offspring. The results indicate that female rats receiving the subtoxic dose from day 1 to day 6 showed no significant differences in the number of corpora lutea, implantation sites, or live fetuses per dam when compared to the control group. Similarly, the mean weight and cranio-caudal measurements of the fetuses were comparable between the subtoxic dose group and the control group. However, the post-implantation survival rate in the subtoxic dose group was higher than that of the control group, though it remained consistent with historical control data (HCD). A comparison of the control group to HCD revealed a marked decrease in the post-implantation survival rate **(Table 2)**.

Table 2. The impact of the experimental solution.

Groups and doses	Corpora letua	Implantation sites	Live foetuses	Pre-implantation survival rate (%)	Post-implantation survival rate (%)	Foetus weight (g)	Foetus size (mm)
Days 1-6							
HCD (intact)	11.85 ± 0.37	10.38 ± 0.53	9.71 ± 0.61	12.62 ± 4.10	7.52 ± 2.19	2.47 ± 0.06	29.57 ± 0.24
Control group (1050 mg/kg)	12.43 ± 0.31	11.15 ± 0.71	11.07 ± 0.62	10.36 ± 4.45	1.07 ± 0.73*	2.37 ± 0.06	27.42 ± 1.89
Experimental group (1550 mg/kg)	11.47 ± 0.46	10.87 ± 0.65	9.93 ± 0.73	6.63 ± 3.18	9.60 ± 2.42*	2.49 ± 0.46	30.06 ± 0.29
Days 6-16							
HCD (intact)	11.85 ± 0.37	10.38 ± 0.53	9.71 ± 0.61	12.62 ± 4.10	7.52 ± 2.19	2.47 ± 0.06	29.57 ± 0.24
Control group (1050 mg/kg)	11.47 ± 0.62	10.14 ± 0.86	9.50 ± 0.82	13.36 ± 5.70	5.43 ± 2.93	2.56 ± 0.11	30.02 ± 0.19
Test group (1550 mg/kg)	12.39 ± 0.35	11.00 ± 0.82	10.00 ± 0.71	12.12 ± 5.49	7.62 ± 2.10	2.52 ± 0.07	29.79 ± 3.29
Control group (525 mg/kg)	11.11 ± 0.61	10.67 ± 0.58	9.22 ± 0.76	3.78 ± 1.52	14.38 ± 5.76	2.48 ± 0.08	29.70 ± 0.42
Test group (775 mg/kg)	11.79 ± 0.53	10.93 ± 0.62	10.07 ± 0.65	7.86 ± 2.95	10.00 ± 3.42	2.37 ± 0.10	29.21 ± 0.47
Control group (105 mg/kg)	11.87 ± 0.57	11.44 ± 0.67	11.11 ± 0.61	4.25 ± 2.35	3.62 ± 1.46	2.54 ± 0.04	29.92 ± 0.19
Test group (155 mg/kg)	11.07 ± 0.45	10.00 ± 0.62	9.13 ± 0.77	9.33 ± 3.62	10.47 ± 3.49	2.49 ± 0.05	29.69 ± 0.24
Days 16-20							
HCD (intact)	11.85 ± 0.37	10.38 ± 0.58	9.71 ± 0.61	12.62 ± 4.10	7.52 ± 2.19	2.47 ± 0.06	29.57 ± 0.24
Control group (1050 mg/kg)	10.85 ± 0.74	9.23 ± 0.99	8.59 ± 0.95	13.08 ± 7.17	6.92 ± 2.24	2.49 ± 0.05	29.73 ± 0.19
Test group (1550 mg/kg)	11.86 ± 0.54	10.93 ± 0.63	10.00 ± 0.48	8.36 ± 2.34	7.36 ± 2.34	2.37 ± 0.04	28.97 ± 0.66
Control group (525 mg/kg)	11.89 ± 0.54	11.11 ± 0.70	10.00 ± 0.67	6.33 ± 4.59	11.11 ± 0.97	2.49 ± 0.04	29.96 ± 0.22
Test group (775 mg/kg)	11.00 ± 2.57	10.70 ± 0.65	9.40 ± 0.78	5.40 ± 3.13	9.30 ± 3.23	2.51 ± 0.06	30.73 ± 0.79

The experimental doses, including the subtoxic and intermediate levels given during the organogenesis and growth stages (from day 6 to day 16 and day 16 to day 20), along with the therapeutic dose from day 6 to day 16, did not result in any reduction in the number of corpora lutea, implantation sites, or viable fetuses. Upon conducting a thorough macroscopic analysis of 1610 fetuses from all groups, no external malformations were found. However, the rats receiving the subtoxic dose between days 1-6 and days 6-16 showed a higher frequency of subcutaneous hemorrhage, with up to twice as many affected fetuses compared to those in the control and historical control groups ($P < 0.05$). In contrast, no notable differences in the occurrence of subcutaneous hemorrhage were seen in rats treated with the subtoxic and intermediate doses between days 16-20 ($P > 0.05$).

To examine possible internal abnormalities, the Wilson technique was employed to assess 805 fetuses. No significant variations in the incidence of visceral malformations were observed between the control group, historical controls, or test groups treated with the subtoxic dose from days 1-6 or days 16-20, and those receiving the intermediate or therapeutic doses from days 6-16. In some cases, a notable decrease in the rates of hydrocephalus and hydronephrosis was recorded compared to the control and historical control groups. Notably, in the test group treated with the subtoxic dose **(Table 3)**.

Table 3. The data on foetal abnormalities.

Group	Dose	Period (days)	Number of foetuses (%)			
			Subcutaneous haemorrhage	Cholestasis	Hydrocephalus	Hydronephrosis
HCD	-	-	39.1	43.4	7.2	10.4
Control group	1050	1-6	19.1	30.1	20.5	2.7
Test group	1550	1-6	20.3	29.7	7.8	1.6

Control group	1050	6-16	17.3	39.1	13.0	0.0
Test group	1550	6-16	21.0	54.7	14.7	5.2
Control group	525	6-16	43.9	34.1	12.1	2.4
Test group	775	6-16	38.8	27.7	0.0	0.0
Control group	105	6-16	43.0	37.2	13.1	1.9
Test group	155	6-16	28.0	42.0	15.8	2.8
Control group	1050	16-20	27.5	20.0	18.7	8.7
Test group	1550	16-20	43.4	28.0	2.1	8.7
Control group	525	16-20	40.0	27.0	9.8	13.7
Test group	775	16-20	39.0	21.8	18.7	1.5

A detailed examination of 788 fetuses' skeletal development revealed differences in the rate of bone ossification. Fetuses from dams receiving the subtoxic dose throughout pregnancy displayed faster ossification of key skeletal areas, such as the sternum, sacrum, and wrist ($P < 0.05$). The accelerated bone development was also observed when the test solution was given between days 16 and 20, with the effect continuing even at a reduced dose.

In contrast, when intermediate and therapeutic doses were administered during the critical phase of organogenesis, fetuses exhibited delayed ossification in several bones, including the sternum, metatarsus, and sacrum ($P < 0.05$). Similarly, the control solution containing glucose and ascorbic acid resulted in a slower ossification process in fetuses of treated dams, with significant differences noted in the sternum and sacrum compared to historical control data. However, a reduction in the dose of the control solution led to faster ossification in these same regions. Fetuses from dams administered lower doses of glucose and ascorbic acid also showed enhanced ossification of various bones compared to the historical control group ($P < 0.05$).

No significant discrepancies in appearance, behavior, or weight were detected between the group of rats treated with 1550 mg/kg of the test solution from Days 6 to 22 and the control rats **(Table 4)**. Both groups exhibited a slower weight gain when compared to historical controls.

The pups were born on time, and their body weight changes were monitored for up to 60 days. Female pups from the test group gained more weight than those from the control group, although both groups' weight gain was in line with the historical control values. It is worth mentioning that at 15 and 30 days, pups from both the test and control groups were lighter compared to the historical controls **(Table 4)**.

No significant differences in physical development, such as hair growth, incisor eruption, eye-opening, or testicular descent, were noted across the groups. However, the control group's female pups showed a delayed onset of vaginal opening (5 days later on average) compared to those from the experimental group.

Behavioral assessments for motor skills were carried out on five-day-old pups, including tests like the horizontal rope walking test, righting reflex, and cliff avoidance, with not much difference between the test and control groups **(Table 4)**.

Further testing for muscular strength in 15-day-old pups included grip strength and pull-up tests, evaluating their forelimb and hind limb strength and endurance on a bar **(Table 4)**.

Lastly, at one month, an open field test was performed to analyze exploratory behavior, including crossing, rearing, and grooming patterns **(Table 4)**.

Table 4. The dynamics of body weight change during pregnancy.

Group	**Days 1-7**	**Days 7-14**	**Days 14-22**	**Days 1-22**
HCD	31.00 ± 2.45	30.00 ± 2.47	49.00 ± 6.00	110.00 ± 7.58
Control group	23.00 ± 3.73	19.17 ± 0.83*	42.50 ± 4.23	85.83 ± 6.76*
Test group	26.00 ± 1.87	15.00 ± 1.58**	49.00 ± 2.92	90.00 ± 3.54**

The dynamics of body weight change of the pups (g)

Group	Sex	Pups' age, days			
		5	15	30	60
HCD	Female pups	9.48 ± 0.20	28.12 ± 1.11	64.15 ± 2.18	155.82 ± 4.91
	Male pups	9.71 ± 0.23	29.04 ± 2.53	66.66 ± 5.38	157.88 ± 7.09
Control group	Female pups	8.68 ± 0.56	22.70 ± 1.30*	55.05 ± 2.90*	136.48 ± 5.44*

	Male pups	8.79 ± 0.46	22.55 ± 1.02*	56.46 ± 2.37*	140.15 ± 5.74
Test group	Female pups	8.96 ± 0.53	22.11 ± 1.47±**	55.76 ± 1.15**	164.00 ± 4.97*
	Male pups	9.26 ± 0.61	19.37 ± 1.21**	54.51 ± 1.71**	152.566 ± 4.83

The integration of the sensory and motor systems of the five-day-old pups (x ± m)

Group	Sex	Horizontal rope walking test (% of pups)	Horizontal rope walking test length (sec.)	Developed cliff avoidance response (%)	The righting reflex (sec.)
HCD	Female pups	100.00	8.25 ± 1.44	65.00	12.85 ± 2.82
	Male pups	100.00	7.80 ± 1.51	61.60	8.12 ± 1.44
Control group	Female pups	90.33	10.08 ± 1.64	69.50	9.46 ± 3.90
	Male pups	95.83	10.07 ± 1.74	55.50	5.67 ± 2.04
Test group	Female pups	100.00	9.68 ± 1.05	53.40	5.78 ± 1.74
	Male pups	100.00	9.50 ± 0.36	35.00	4.75 ± 0.86

The integration of the sensory and motor systems of 15-day-old pups (x ± m)

Group	Sex	Number of pups grasping with forelimbs (%)	Hang time with forelimbs (sec.)	Number of pups grasping with hind limbs (%)	Number of pups able to perform pull-ups (%)
HCD	Female pups	100.00	30.63 ± 10.06	100.00	100.0
	Male pups	100.00	30.83 ± 12.29	100.00	100.00
Control group	Female pups	100.00	9.86 ± 1.49*	91.67	86.17
	Male pups	100.00	10.54 ± 2.25*	73.67	83.33
Test group	Female pups	100.00	18.54 ± 3.11*	100.00	100.00
	Male pups	100.00	21.25 ± 2.54*	100.00	100.00

The open field test of one-month-old pups (x ± m)

Parameter	HCD		Control group		Experimental group	
	Male pups	Female pups	Male pups	Female pups	Male pups	Female pups
Line crossing	40.95 ± 3.30	49.47 ± 6.86	39.60 ± 3.02	38.59 ± 3.02	48.02 ± 3.76	44.88 ± 6.51
Hole board	5.60 ± 0.90	6.10 ± 1.67	6.54 ± 0.81	7.30 ± 1.01	7.62 ± 0.94	6.47 ± 1.10
Rearing	4.20 ± 1.14	7.10 ± 1.20	5.72 ± 0.98	7.49 ± 4.09	8.53 ± 1.20	3.55 ± 0.65
Grooming	0.45 ± 0.12	0.65 ± 0.23	0.96 ± 0.04	0.96 ± 0.19	0.67 ± 0.15	0.78 ± 0.15
Defecation	1.85 ± 0.38	2.10 ± 0.36	3.07 ± 0.30	2.79 ± 0.61	2.38 ± 0.15	2.56 ± 0.21

To evaluate the learning and memory capabilities of the pups, the passive avoidance test was conducted. On the first day, the pups were allowed to explore both light and dark compartments, and the time spent in each compartment was recorded. The results revealed that the pups from the test group showed reduced learning ability **(Table 5)**. Interestingly, the control group also exhibited a lower level of learning performance compared to the historical control data.

The pups' stress responses were assessed by observing active behaviors such as swimming, diving, and climbing. The findings revealed a significant difference in stress responses when compared to the historical control data. However, the defecation rates in both the control and test groups were notably lower than those observed in the historical control group **(Table 5)**.

Table 5. The passive avoidance response.

Parameter	**HCD**		**Control group**		**Experimental group**	
	Female pups	**Male pups**	**Female pups**	**Male pups**	**Female pups**	**Male pups**
1 Day 1						
Burrowing (%)	100.00	100.00	100.00	100.00	100.00	100.00
Time spent in a lit compartment (sec.)	22.00 ± 4.15	64.60 ± 10.64	22.08 ± 3.98	49.33 ± 13.48	27.33 ± 5.04	39.00 ± 9.41

Time spent in a dark compartment (sec.)	158.00 ± 4.15	115.40 ± 10.64	157.92 ± 3.98	130.67 ± 13.48	153.67 ± 5.04	141.00 ± 9.41
Day 2						
Number of pups with developed passive avoidance response (%)	90.00	100.00 ± 0.00	66.70	75.00*	33.33**	44.44**
Time spent in a lit compartment (sec.)	163.50 ± 16.50	180.00 ± 0.00	127.00 ± 22.75	135.08 ± 23.46	99.44 ± 22.55**	118.78 ± 24.50**
Time spent in a dark compartment (sec.)	16.50 ± 16.50	0.0	53.00 ± 22.75	44.92 ± 23.46*	80.56 ± 22.55**	61.22 ± 24.50**

The active stress response

Parameter	HCD		Control group		Test group	
	Male pups	Female pups	Male pups	Female pups	Male pups	Female pups
Number of diving pups (%)	80.0	90.0	70.0	28.57*	72.8	60.0
Time spent in an inner cylinder (sec.)	81.25 ± 20.85	63.00 ± 13.22	51.14 ± 23.58	63.75 ± 23.57	35.00 ± 14.08	44.88 ± 6.51
Time spent in an outer cylinder (sec.)	17.38 ± 5.76	13.00 ± 7.08	8.86 ± 3.59	16.75 ± 3.57	10.50 ± 2.67	11.50 ± 2.58
Climbing (mesh) (sec.)	2.50 ± 0.33	11.00 ± 6.14	3.43 ± 1.19	2.50 ± 0.50	2.12 ± 0.23	3.00 ± 0.63
Defecation	4.25 ± 0.75	2.56 ± 0.75	1.71 ± 0.57*	1.75 ± 0.63	1.38 ± 0.42*	0.83 ± 0.17

The assessment of fertility and both pre and post-implantation mortality involved comparing key indicators such as corpora lutea, implantation sites, and the number of live or dead foetuses. The application of a subtoxic dose of the experimental solution for 15 consecutive 24 hours before mating with intact males showed no substantial impact on these parameters **(Table 6)**.

Additionally, no significant changes in survival rates or weight gain were found from the administration of the subtoxic dose. Furthermore, no delays in puberty were observed in the groups treated with either the intermediate or therapeutic doses.

When the same subtoxic dose was administered before mating with intact females, no adverse effects on litter outcomes were noted. The survival rates, physical growth, and weight gain of the pups were comparable to those in the control group. Analysis of testicular morphology revealed no significant differences between the groups when compared to the historical control data **(Table 6)**.

Table 6. The subtoxic dose of the experimental solution and the fertility: females (x ± m)

Parameter	**Group**		
	HCD	**Control group**	**Test group**
Corpora lutea per female	12.55 ± 0.58	12.42 ± 0.57	13.36 ± 0.59
Implantation sites per female	12.00 ± 0.70	11.58 ± 0.60	12.18 ± 1.19
Live foetuses per female	7.20 ± 3.12	11.25 ± 0.60	11.64 ± 1.19
Pre-implantation mortality (%)	10.09 ± 0.91	6.66 ± 2.55	11.04 ± 6.33
Post-implantation mortality (%)	13.82 ± 4.86	3.33 ± 0.15	5.05 ± 1.84
Fertility index (%)	76.2	100.0	89.5
Pregnancy index (%)	100.0	100.0	85.0

The subtoxic dose of the experimental solution and the fertility: males (x ± m)

Parameter	Group		
	HCD	Control group	Test group
Corpora lutea per female	12.55 ± 0.58	13.33 ± 0.37	13.22 ± 0.80
Implantation sites per female	12.00 ± 0.70	11.89 ± 1.30	12.67 ± 0.83
Live foetuses per female	7.20 ± 3.12	11.33 ± 1.29	11.56 ± 0.69

Pre-implantation mortality (%)	10.09 ± 0.91	11.11 ± 9.30	4.00 ± 3.04
Post-implantation mortality (%)	13.82 ± 4.86	4.33 ± 2.61	8.00 ± 3.37
Fertility index (%)	76.2	83.3	63.7
Pregnancy index (%)	100.0	93.0	100.00

The data from this study reveal that administering the subtoxic dose of the test solution from days 16 to 20, along with the therapeutic dose from days 6 to 16 of pregnancy, resulted in a reduction of weight gain during specific stages of pregnancy. Rats that received the subtoxic dose during implantation showed an increase in post-implantation mortality. Additionally, a higher frequency of foetuses with subcutaneous hemorrhage was observed when the subtoxic dose was given during implantation and organogenesis.

Bone ossification was influenced by pantohematogen, with the subtoxic dose accelerating bone formation, especially during fetogenesis. However, foetuses exposed to the intermediate and therapeutic doses during organogenesis exhibited slower ossification.

Regarding the pups' postnatal development, those born from dams injected with the subtoxic dose from days 6 to 22 showed no signs of developmental delays. At two months, the pups from the test group had a higher body weight than those from the control group. Behavior tests, such as the open field test, showed similar results across all groups.

The learning performance of the pups in the experimental group was similar to that of the control group, but both had lower abilities than the HCD group. When assessing stress responses, all pups demonstrated appropriate reactions to stress, but those in the experimental group displayed lower anxiety compared to the control pups.

Conclusion

The results of this study suggest that pantohematogen does not influence fertility or contribute to an increase in embryonic mortality. Additionally, no delays in puberty onset were observed at lower doses. Overall, the findings indicate that pantohematogen does not exhibit any harmful embryotoxic or teratogenic effects.

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